FEDERAL EXPERIMENT STATION IN PUERTO RICO

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CINCHONA PROPAGATION

By

HAROLD F. WINTERS, *Horticulturist*

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MAYAGÜEZ, PUERTO RICO

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Federal Experiment Station in Puerto Rico

CONTENTS

INTRODUCTION

Cinchona, the natural source of quinine, is a tree native to the South American Andes from Venezuela to Bolivia and to certain mountainous sections of Central America. Quinine and related alkaloids extracted from cinchona bark, have valuable medicinal properties and quinine, in particular, is specific for the treatment of malaria. The value of cinchona bark in the treatment of malaria was known in Peru prior to 1633, but the facts about its discovery are probably lost to history (*31*).[1]

In recent years, various synthetic drugs have been found to have antimalarial properties but none has proved as safe as natural quinine. Synthetics may be injurious to health unless administered under the direction of a competent doctor and are, therefore, unavailable to millions of people who suffer from malaria due to complete lack of medical care if not because of the original cost.

Cinchona culture is limited by its growth requirements to relatively cool, frost-free areas having rich, deep, well-drained and aerated soil

[1] Italic numbers in parentheses refer to Literature Cited, p. 25.

and high rainfall. Such an environment is found in relatively limited mountainous areas of the Tropics.

For over 200 years after the discovery that infusions of bark from the cinchona tree would cure malaria, no attempt was made to cultivate the trees. The world supply of bark was harvested from natural stands in the Andes region of South America.

As the natural supply dwindled, officials of several European countries began to fear that the supply might become exhausted and persuaded their governments to act on the matter. As a result many attempts were made to secure propagation material from South America and transport it to other tropical areas, particularly the colonies of Holland and Great Britain. Accounts of these efforts (*17, pp. 13–34*) are as romantic and exciting as fiction.

HISTORY

Since about 1850 cinchona culture has been attempted in practically every tropical and many subtropical countries. The early experiments were largely discouraging, but the most successful results were secured in Ceylon, India, and Java.

Early attempts by the Dutch Government to cultivate cinchona in Java are described in detail by Moens (*17, p. 21*). The first introduction of cinchona in Java was a specimen of *Cinchona calisaya* Wedd.,[2] which was obtained through exchange with the florists, Thibaut and Keteleer in Paris. The plant was grown from seed brought from Bolivia by Weddell. Other experiments followed as seed or plants could be secured from South America.

The most fortunate introduction of *Cinchona* to Java was the small lot of seed which the Netherlands Government obtained from Charles Ledger in 1865 (*17, p. 22*). The strain which originated from this seed later became known as *C. ledgeriana* Moens. Because of its high yield of alkaloids the Dutch planters were able to survive the low prices of the overproduction period which occurred around 1890 and to eventually become the major source of world supply.

According to Cowan (*3, pp. 46–47*), the development of cinchona culture by the Dutch in Java was paralleled by trials instigated by the British in India, Burma, Ceylon, Malaya, the Sudan, St. Helena, Jamaica, Trinidad, Mauritius, Australia, and New Zealand. The efforts were most successful in Ceylon and South India where private planters, encouraged by the success of government ventures, took up the cultivation of cinchona. By 1890 world production of cinchona bark was considerably in excess of demand and prices fell. Subsequently, most private cinchona growers uprooted their plantations and put in tea. At present practically no cinchona plantations are reported in these countries other than those owned and managed by the governments.

Toward the end of the last century President Justo Rufino Barrios of Guatemala and others became interested in cultivating cinchona in Central America (*22, pp. 308–309*). From the results of their trials a new cinchona industry has sprung up there which seems likely to become an important factor in world quinine production.

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[2] *Cinchona calisaya* Wedd., *C. ledgeriana* Moens, and others are considered to be varieties of *C. officinalis* L. by Standley (*28, p. 272*), but the author prefers to follow the nomenclature in more common usage to avoid confusion.

Recorded experimentation on cinchona culture is almost nonexistent, but considerable has been written on the subject generally. Pertinent literature will be discussed under the various section headings. For a complete annotated bibliography on the subject since 1883 the reader is referred to Moreau (*18*).

HISTORY OF CINCHONA IN PUERTO RICO.—The first record of *Cinchona* in Puerto Rico was a planting made by seed at the United States Forest Service nursery at Rio Piedras in 1925 (*2, p. 564*). At that time seedlings of *C. ledgeriana* and *C. succirubra* Pavon [3] were grown to a height of about 4 decimeters. The first successful field planting was made by the Forest Service in the Maricao Insular Forest in 1933 (*25, p. 91*). At that time 839 seedling trees were imported from the United States Plant Introduction Garden of the Bureau of Plant Industry, Glenn Dale, Md.

In 1935, the Federal Experiment Station in Puerto Rico undertook a research program to study the various phases of cinchona culture and assumed responsibility for the original Maricao planting. In June of that year, 1,000 additional cinchona seedlings representing 17 species or hybrids of species were received from the United States Pant Introduction Garden. These seedlings were planted at Maricao and at two new locations, the Las Mesas property of the Federal Experiment Station, and Doña Juana in the Toro Negro National Forest.

The highest survival and best growth was obtained at Maricao, at a location on the leeward or southwest slope of a mountain at an approximate elevation of 2,600 feet above sea level. The Las Mesas planting at 1,000 feet elevation with good wind protection grew well at first but most of the trees died later. The poorest results were obtained at Doña Juana. This location at 3,000 feet elevation had no wind protection and the soil was thin and low in fertility. After 1 year the few surviving trees were moved to Maricao. In 1939 a nursery was established at Maricao and this became the center of cinchona investigations in Puerto Rico for the next few years.

By 1941 it was apparent that the results being obtained at Maricao were not satisfactory, although several thousand seedlings were produced and planted in nursery beds. Another location at higher elevation was selected in the Toro Negro National Forest. This area, located at an elevation of 3,000 to 4,000 feet in a valley between two mountain ranges has better protection from the prevailing winter winds at a time when rainfall is low. Annual rainfall is estimated at 100 inches and annual mean temperature at 67.3° F. The land was at one time a coffee plantation but has not been cultivated as such since 1928. Although clay soils predominate in the high areas of Puerto Rico, this particular section contains areas of loamy soils derived from granite and related volcanic materials. This was one of the important factors considered in selecting the site. Results at this location have been more encouraging than previous trials.

PROPAGATION METHODS

Cinchona can be propagated by seed, cuttings, or grafting. Choice of method depends largely upon economic factors and the purpose for which the plants are desired. For example, propagation of the high-

[3] Botanical names are as given in the literature reference cited.

yielding Ledger strains by cuttings has proved too difficult for production of large numbers of plants for field planting. On the other hand, comparatively easy techniques have been developed for producing large numbers of seedlings and grafted plants. Whether seedlings or grafted plants are used for field plantings, the seedling practices are equally important. In grafting it is necessary to have available large numbers of *Cinchona succirubra* rootstocks which are produced easily and cheaply from seed.

In Java (*17, p. 149*) the first attempts to grow plants from South American seed failed because of lack of knowledge concerning the requirements for germination and early growth. The first planting in the open ground was a complete failure. Later a few plants were secured when seed was sown in clay pots or hollow bamboo sections plunged in covered nursery beds or kept partially submerged in tubs of water in greenhouses. As the older trees matured and larger quantities of seed became available the practice of sowing in germination houses built of bamboo came into use. At first, considerable attention was given to propagation by cuttings, but this practice was later supplanted by propagation from seed. Sands (*27*) describes these germination houses in detail as well as the transplant nurseries which were commonly used in Java and have now come into common use wherever *Cinchona* is cultivated.

Propagation by Seed

Seed characteristics

Seed of the genus *Cinchona* are flat with an oblong nucleus which is surrounded by a papery wing with slightly dentate margins. The embryo forms the axis of a pulpy albumen. It is possible to separate good seed from bad on a frosted pane of glass which is lighted from beneath to show the presence or absence of the embryo. The seed of the different species differ in size and shape. According to Moens (*17, p. 148*), those of *C. officinalis L.* are 4 to 7 millimeters long and 2 to 3 millimeters wide; of *C. ledgeriana* 4½ millimeters long and 1 millimeter wide; and those of *C. succirubra* 7 to 10 millimeters long and 2 to 3 millimeters wide. Representative seed of some *Cinchona* spp. may be seen in figure 1,*A*. The seed are extremely light in weight; 1 gram contains 2,000 to 3,000 *C. ledgeriana* seed (*22, p. 313*).

SEED STORAGE.—To have maximum viability cinchona seed should be harvested when well ripened and dried in the air for several days before cleaning of all capsule parts and placentae. When stored in a tight tin box such seed remain viable for 14 months or longer (*17, p. 148*). Kerbosch (*11*) reported that cinchona seed can be kept for 5 years at humidity below 50 to 60 percent. These conditions were obtained by storage over calcium chloride (fig. 1,*B*).

The chemicals and chemical compounds recommended in the following pages, particularly those under "Diseases and Insects," pp. 19–24, are all more or less poisonous. Some are highly flammable; some are caustic and corrosive. Persons unfamiliar with the dangers encountered in the handling, use, and storage of such chemicals should secure expert advice before attempting their use.

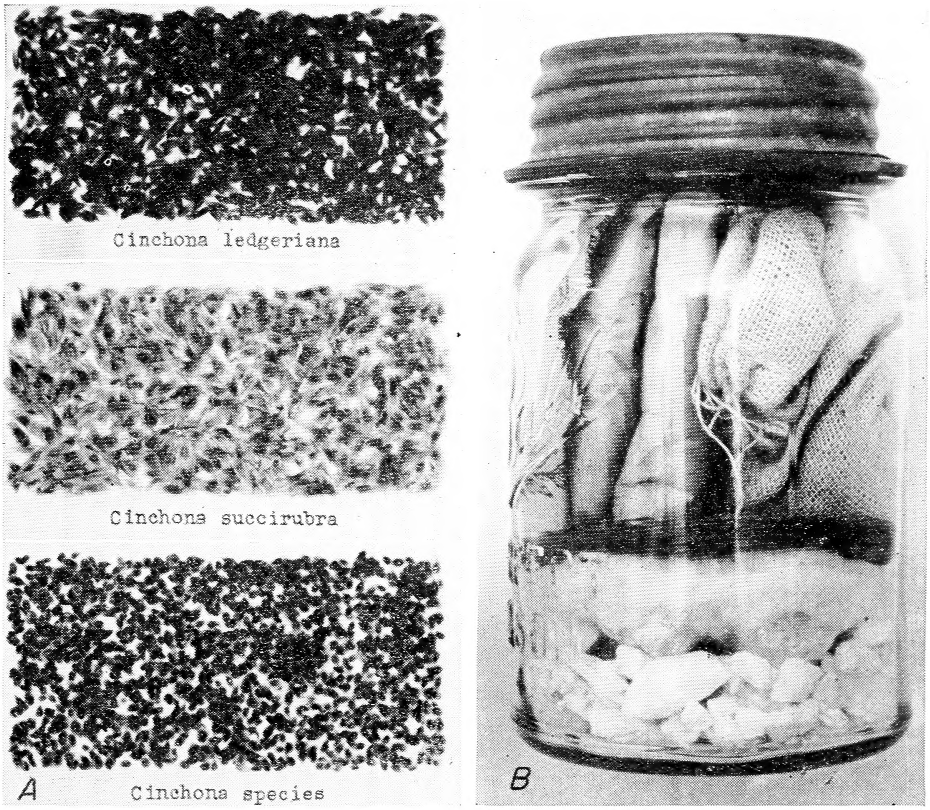

FIGURE 1.—*A*, From top to bottom are shown 60 percent natural size, representative seeds of *Cinchona ledgeriana* Moens, *C. succirubra* Pavon, and an unidentified species of *Cinchona*. *B*, Glass jars with screw or clamp tops can be used to store cinchona seed. The layer of calcium chloride is covered with cotton and blotting paper to prevent contact with the seed.

Cowgill (*4, pp. 6–19*) showed that both humidity and temperature are extremely important factors in survival of stored cinchona seed. The safest range was between 33 and 66 percent humidity and 7° and 24° C. He also demonstrated that storage of *Cinchona ledgeriana* seed in daylight caused more rapid deterioration than storage in the dark (*4, p. 20*). This was particularly true where humidity was maintained sufficiently high to approach favorable germinating conditions.

Germination factors

LIGHT.—In all reports on the technique of growing *Cinchona* from seed, it is recommended that the seed be planted on the surface of the seedbed or, if they are covered, that the covering be very thin. Light is apparently necessary to start the germinative processes within the seed even when other conditions are favorable. Working at the United States Plant Introduction Garden, Cowgill (*4, pp. 27–28*) demonstrated that cinchona seed sown under conditions otherwise favorable for germination did not germinate when held in complete darkness for 21 or 50 days. When brought into the light, seed planted for both 21 and 50 days reached maximum

germination in an additional 21 days. This is the usual time required for freshly planted seed.

MOISTURE.—Attention to moisture is particularly important in the germination of cinchona seed because the seed must be exposed to light on the soil surface and not buried in the seedbed medium as with most seeds. An adequate moisture supply must be maintained because, once the germinative process has started, drying will cause death. On the other hand, excess water may cause rotting (*17, p. 150*).

TEMPERATURE.—Moens (*17, p. 150*) considered a temperature of 18° to 24° C. necessary for best germination of cinchona seed. In a more carefully controlled experiment, Cowgill (*4, pp. 37–45*) found that *Cinchona ledgeriana* seed germinated over a range of temperatures from 14° to 35° C. but the optimum temperature was between 24° and 29.5° C., with an extremely rapid loss in germinability at temperatures above 29.5° C. These findings may well be used as a guide to the proper season for sowing seed in regions with low winter or high summer temperatures.

Considering these factors it is obvious that in order to insure germination of the cinchona seed and good growth of the seedlings considerable control of environmental conditions is necessary. The seedbeds and nurseries described in this bulletin were designed primarily for this purpose.

Cinchona seedbeds

LOCATION.—Selection of sites for cinchona seedbeds should receive detailed consideration in advance of construction. The site should be level or sloped gently to the north. Protection from wind is essential in areas where strong winds may occur at some seasons. On the other hand, good air drainage is also essential to prevent excessive dampness. For this reason seed nurseries located in small valleys have given good results. Forest clearings assure good light conditions, but no trees should be left standing in the nursery area as the roots have a tendency to penetrate the seedbed medium and compete with the young cinchona plants for moisture and nutrients.

It is essential that the seedbeds be placed near a stream or other source of water as all watering is preferably done artificially. It is an additional advantage when locating the seedbeds to select a site in the vicinity of the transplant nurseries. This will save time later in transplanting and result in less damage to the plants.

In the Northern Hemisphere the front or open side of the seedbed shelter should face north or slightly northeast; south of the Equator orientation would be in the opposite direction. Such orientation will obviate the possibility of direct sunlight striking the seedbed most of the year. The open front as described will allow entrance of sufficient light for early growth of the seedlings. Other considerations such as direction of prevailing winds would probably be more important near the Equator.

SHELTER CONSTRUCTION.—Various types of shelters have been used successfully in growing cinchona seedlings. Actually any structure may be used in which the proper conditions of light, temperature, soil, moisture, and humidity can be supplied. Choice of type will depend upon the materials available, the climate, and the preference of the

operator. Sands (*27*) describes a temporary seedbed shelter built of bamboo poles and thatched with "ilang-ilang" grass (*Imperata arundinacea* Cyr.). Temporary shelters are sometimes used in Guatemala as may be seen in figure 2,*B* and *C*, but the more permanent type is also used (fig. 2,*D* and *F*). In Costa Rica a permanent-type shel-

FIGURE 2.—Various types of shelters have been used successfully in growing cinchona seedlings. *A*, Permanent-type shelters with a front span of thatch which can be removed to admit more light when the seedlings are several months old. *B*, Seedbeds of *Cinchona ledgeriana* sheltered by hand-split panels of cypress wood. In this case there is no control of humidity so seeding is done during the rainy season. *C*, Semi-permanent shelters which are covered at planting time with muslin cloth, being used for nurserybeds when photographed. *D*, Permanent seedbed shelters covered with composition roll roofing and on the extreme right by cypress shingles. *E*, Damage caused to a germinating cinchona seedbed by direct sunlight. *F*, Excellent growth of *C. succirubra* obtained in the permanent-type seedbeds shown in *D*.

ter was seen (fig. 2,*A* and *E*) with a front span that could be removed to allow greater entrance of light. Some of these permanent arrangements have been used for a number of years with good results.

In Puerto Rico a permanent-type shelter has been developed which

apparently provides near optimum environmental conditions for germination and early growth. A group of these shelters each 50 feet in length is shown in figure 3. These sheds are 5 feet wide and may be as long or short as the terrain will allow. All lumber should be creosote-coated to make it more durable in contact with the soil. The uprights of small hewn logs or 2- by 6-inch sawed lumber are imbedded in concrete and stand 6½ feet high at the ridge, from which the roof slopes sharply to the back where the eaves are 2 feet above the ground. The 2½-foot front span is 6 feet high at the eaves. Sawed 2- by 4-inch

FIGURE 3.—Permanent shelter houses roofed with zinc have been used to advantage in Puerto Rico for protecting cinchona seedbeds. Duck- and tobacco-shade cloth was used to regulate light at time of seeding, and a covering of palm leaves was placed over the metal roofing to prevent heating in the sun.

lumber is used for rafters and braces and 1- by 4-inch boards support the roofing as shown in figure 4,*A*. Seven-foot-lengths of galvanized iron are used as roofing material.

Each shelter house protects a single 3-foot-wide seedbed which fits snugly between the center and the rear posts and is as long as the shelter house. An average size man can stand upright and walk in the passageway at the front of the seedbed. The sides of the seedbed are of ½- or ¾-inch creosoted lumber 6 inches wide.

Seedbed management

SEEDBED SOIL.—In preparation for seeding, the seedbeds are filled with a medium which will provide the proper moisture for germination and furnish the necessary nutrients to the young plants after germination. In addition it must be well aerated and well drained as disease develops quickly in a water-logged soil. For this purpose Sands (*27*), Blücher (*1*), Meijlink (*16*), and Groothoff (*8, p. 21*)

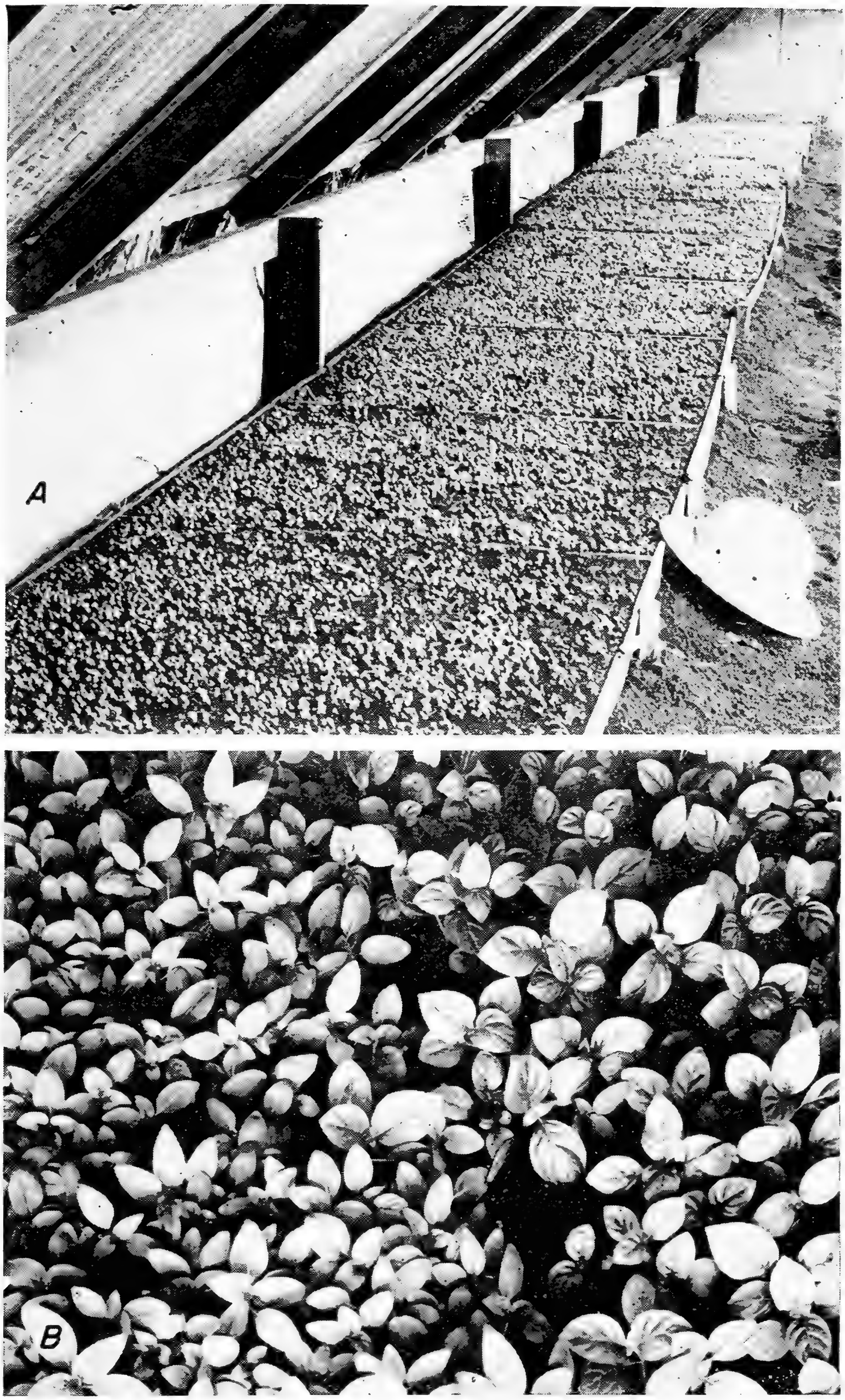

FIGURE 4.—*A*, Cinchona seedbed a few weeks after germination; note construction details of the shelter house. *B*, Seedlings of *Cinchona ledgeriana* (left) and *C. succirubra* (right) ready for transplanting to the nurseries.

report the use of pure humus in the East Indies. Fresh jungle mold is collected from the virgin forest floor and screened to remove all stones, insects, live roots, and other material. Detmer (*7, p. 57*) states that seed are sown on rich black soil, high in humus, free of sand, and not too fine in structure. On the other hand, Stockdale (*29*) states that soil must contain a fair proportion of sand and finely divided organic matter. Popenoe (*23, p. 28*) also recommends the use of rich forest soil mixed with 25-percent clean sand.

In an experiment conducted at this station (*10, pp. 17–20*) the best germination and growth of cinchona seedlings was secured with a mixture of fresh humus, sand, and composted cow manure in the ratio of 1 : 2 : 1. A liquid obtained by soaking 1 pound of dried cow manure in 3 gallons of water has also stimulated growth of cinchona seedlings when sprinkled over the seedbeds. Composted animal manure is also mixed with the seedbed soil at some of the Guatemalan cinchona plantations.

Kevorkian (*12, p. 10*) reports an experiment in which sphagnum moss, peat moss, forest mulch, and sand were tested as germination media. Twenty-eight days after planting the peat moss was outstanding in production of plants, but by the end of the eleventh week sphagnum moss had produced 78.8 percent more seedlings than peat moss and sand and 118.5 percent more seedlings than forest mulch. The decrease in stand after germination was due to disease. Further investigations by Cowgill (*4, pp. 22–26*) showed that the medium was not so important in germination, but that there was a high correlation between medium used and early survival from fungal attack. Greatest losses were in soil and peat with best survival in sphagnum.

Morrison (*19*) reports that sphagnum moss has proved satisfactory as a medium for germination of seed and early growth of the seedlings. It was used extensively at the plant propagation station of the Bureau of Plant Industry, Glenn Dale, Md., during the war, for germinating and growing thousands of cinchona seedlings. These seedlings were later distributed to cooperators in Central and South America for field planting. Plants grown beyond the early seedling stage in a sphagnum medium, however, soon became stunted unless additional nutrients were supplied. The elongated root systems of the seedlings grown in sphagnum probably are subject to more damage upon removal for transplanting than are the more compact root systems of soil-grown seedlings.

A 1-inch layer of sphagnum has been used to advantage as a surfacing material for cinchona seedbeds in Central America. Since the seeds come in contact with the sphagnum only, damping-off is prevented. However, as soon as the roots have penetrated the surface layer of sphagnum to the soil-humus mixture below, proper nutrition is assured.

In Puerto Rico (*33*) a humus-sand mixture with 1-inch surface of screened sphagnum moss effectively prevented damping-off, whereas in adjoining plots of soil-humus the plants were all destroyed by the disease. Treatment of the nursery soil with chloropicrin at the rate of 2 cubic centimeters per square foot before applying the sphagnum greatly increased growth of the seedlings.

Extraordinary caution should be practiced by the user of chloropicrin.

Regulation of Light.—Before seeding it is necessary to reduce the light intensity to the optimum intensity for germination. Kerbosch (*11*) and Cowgill (*4, pp. 27–28*) have shown that some light is necessary for germination of cinchona seed, and Cowgill (*4, p. 29*) established that exposure to 200 foot-candles of light for 10 seconds per day was sufficient to cause almost complete germination in 21 days. However, at this intensity growth was poor and the seedlings remained etiolated after germination. The optimum light intensity for germination is probably between 30 and 75 foot-candles for 6 hours during the brightest part of the day.

In order to produce in the seedbed the conditions described above, it is necessary to reduce the light intensity at the time of seeding. This may be done by tacking white cloth around the open sides of the shelter house, as is shown in figure 3. An effective temporary screen may be made by placing palm leaves along the front and sides of the shelter house. An advantage of this type of screen is that the light intensity can be increased gradually by removing the outer layers first.

Various other devices have been used to reduce light within the seedbed at time of sowing. At one Guatemalan plantation canes or poles were used as is shown in figure 2,*F*. Figure 2,A shows short spans of thatch placed across the front of the shelter houses to prevent direct sunlight from striking the seedbeds as in figure 2,*E*. At another plantation light wooden frames to which cheesecloth had been tacked were placed over the beds after seeding. After germination these frames were raised and placed upright along the sunny side of the seedbed. As soon as the seedlings were well established the frames were removed altogether.

Seeding.—The seed are broadcast evenly over the previously prepared and dampened seedbeds at the rate of 1 to 2 grams per square yard. The actual rate depends upon the viability of the seed and the preference of the operator. Thick sowing will naturally give dense stands of plants which will require transplanting at small sizes.

Before scattering the seed it is best to weigh or measure out the quantity desired for each square yard of seedbed area. The seedbed surface is then marked off into areas of this size and the required amount of seed scattered evenly over each area. Greater uniformity will be secured by this method than if the operator sets out to plant the entire bed.

Usually no covering is given the seed, or only a very thin layer of the seedbed medium is sifted over them. If too much covering is given light will be excluded and germination delayed. However, if plantings are made during dry weather a thin covering will help to maintain dampness around the seed. At this station pulverized treefern root or screened sphagnum moss has been used to advantage as covering for the seed. Instead of covering the seed, they may be lightly raked into the surface of the seedbed.

Watering.—Perhaps the most important single factor in seedbed management is watering, although all phases must be given careful attention. The seedbed media should be thoroughly moistened before the seed are planted. After sowing the seed, heavy watering is

not desirable as the seed are easily washed out of position. The watering method described by Groothoff (*8, p. 22*) has proved useful. This requires a sprayer such as a knapsack sprayer used in vegetable gardens. The seedbed surface is gently moistened with the fine spray after planting and twice each day for 1 week thereafter. During the second week the beds are sprayed once a day. Germination should start by the end of the second week, and subsequent watering is given only as the surface becomes dry.

Too much water on the seed will cause rotting. After the seed have swollen and germination started, too little water will result in death of the seed.

During the first few weeks (fig. 2,*B*) following germination the young seedlings are particularly susceptible to damping-off disease. Too frequent waterings will almost always bring on the disease (fig. 5, *A*), and for this reason it is important to water only as necessary to maintain adequate soil moisture. If the required amount of water is applied in the morning the seedbed surface will be dry by night and this will aid in preventing damping-off. For a more complete discussion of diseases see page 20.

As soon as the roots of the seedlings have penetrated the seedbed medium far enough to support the young plants (fig. 4, *A*) water can be supplied with ordinary watering cans or garden hose. Also, by this time the beds can be wet more thoroughly. Root growth will be increased by letting the surface soil dry out slightly between waterings. This practice will prevent waterlogging and increase aeration of the soil.

WEEDING.—Leafmold gathered from the forest floor may contain considerable quantities of weed seed. These seed usually begin to germinate before the cinchona and if left in the seedbed will crowd the germinating cinchona seed. Weeds usually grow faster than the young cinchona plants which are rather slow growing. It is possible in a few minutes each morning to pull out the weeds which have germinated during the night. If this is done regularly the beds will be clean when the cinchona seed start to germinate. Where sphagnum moss is used as a seedbed medium or for surfacing, weeds are practically eliminated. Fumigation of the soil for disease control as described on pages 10 and 20 will practically eliminate the weed problem.

SEASON.—In the protected seedbeds described in this bulletin, *Cinchona* may be planted at any time of the year with good success. However, best results have been obtained in Puerto Rico from sowings made early in the dry season. The seedlings will then be ready for transplanting to nurseries during the following rainy season when they are most easily established in the nurseries.

When the seedlings reach a height of 3 to 4 inches as shown in figure 4, *B*, they are ready for transplanting. This size is usually reached in 4 to 6 months. If the seedbeds become overcrowded, as sometimes happens when seed germinates better than expected, the larger plants may be removed for transplanting when they are about 2 inches tall.

The seedlings are hardened before transplanting by increasing light. This must be done gradually and should be started as soon as the first true leaves develop. When ready for transplanting the

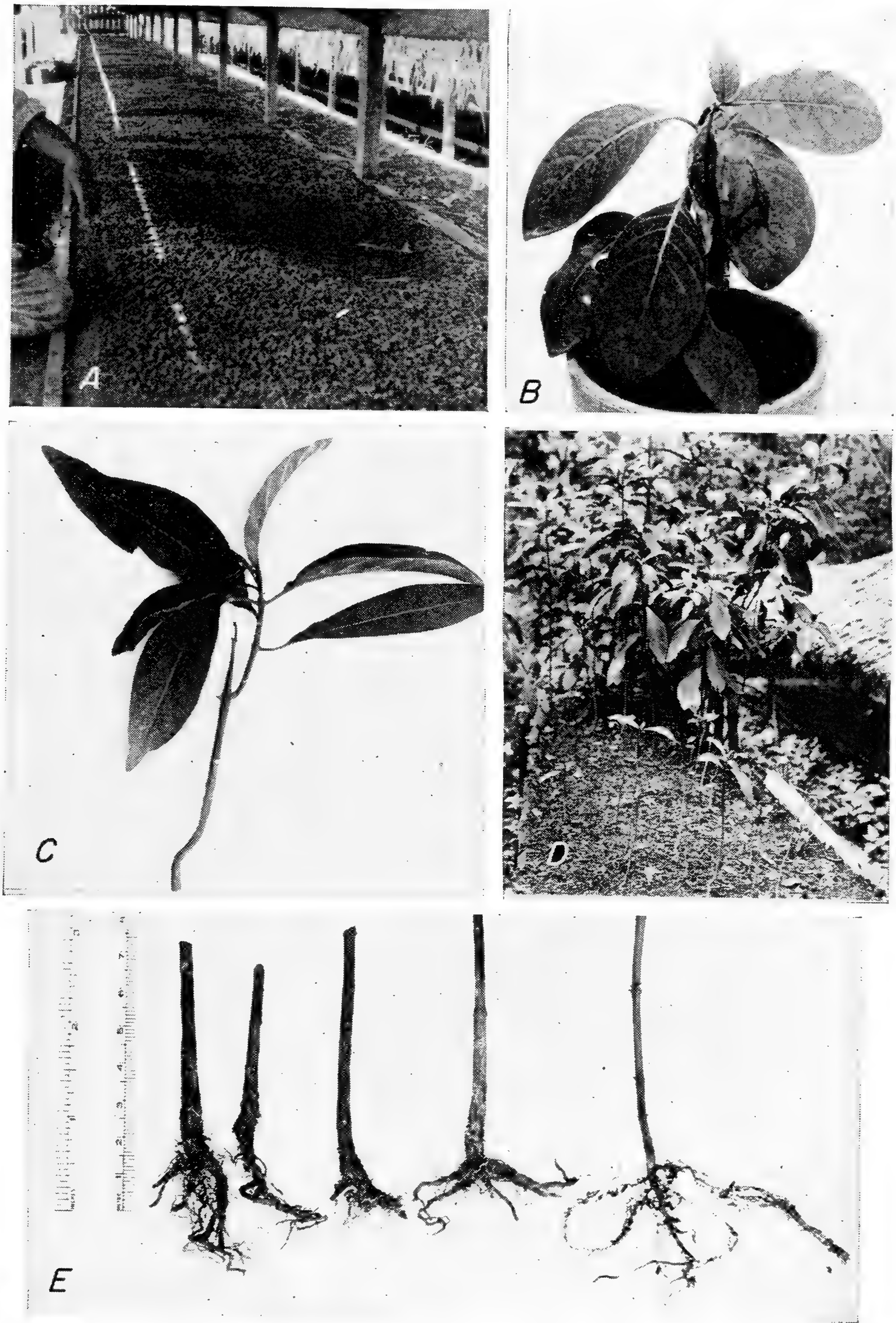

FIGURE 5.—Diseases of cinchona seedlings: *A*, The large spots of damping-off were difficult to control by withholding water during a period of cloudy weather. *B*, Tip blight of young seedlings thought to be caused by a *Phytophthora* sp. *C*, Cinchona plants may recover sufficiently from tip blight to produce new terminal shoots. *D*, Strains of *C. ledgeriana* differ in resistance to a root and collar rot disease. The badly diseased strain in the foreground is from the Philippine Islands while the vigorous strain in the background is the progeny of a tree in the Maricao planting. *E*, Another disease causes the smaller roots to be killed progressively until only stubs remain. Note healthy plant on the right.

plants should receive all possible indirect light and such sunlight as penetrates the seedbed shelter in early morning and late evening.

Transplant nurseries

History.—According to the literature available on the subject, the Dutch cinchona planters use transplant nurseries for growing the young cinchona seedlings when they become overcrowded in the seedbeds. In the early days of cinchona culture in Java, Meijlink (*16*) described two sets of beds which were used for this purpose. The first or intermediate transplant beds were made with "ilang-ilang" grass (*Imperata arundinacea*) roofs so that no rain could reach the plants. The smaller plants were spaced 2 inches apart and larger plants 3 inches. Three to four months later when sufficient growth had been made, alternate rows were transplanted to other nurseries, leaving the remaining plants at 6-inch spacing. These beds were sheltered temporarily by fern leaves. More recently, Sands (*27*) reported that only one transplanting was usually given. Beds were 4 feet wide and 1 foot high, with aisles of 2 feet and were filled with fresh jungle mold. After planting, these beds also were covered closely with a low flat shelter of bamboo and grass or fern leaves, 18 inches off the ground. In Guatemala, similar beds are in use today as is shown in figure 6,*C*. In this case, pieces of wild cane or small poles were used as the shading material. In Costa Rica (fig. 6,*D*), cut elephant grass was used as shading material supported by a low framework of wooden poles.

Selection of Site.—Selection of site is equally as important in locating transplant nurseries as it is in location of seedbeds and the requirements are practically the same. The nurseries should be away from trees but preferably protected from strong winds by forest or hills. A source of water should be at hand as considerable watering may be necessary.

The transplant nurseries should be located on good soil and it is preferable to select sites where *Cinchona* has not been planted before. Nearness to seedbeds is important in order to save labor in transporting plants.

Construction.—In Puerto Rico, nursery beds are constructed similar to those used in Java. The surface of the bed is raised about 1 foot higher than the level of the ground by a retaining wall of palm slats. Wooden posts and poles are used to support a shelter of palm leaves over each bed. As shown in figure 6,*A*, the shelter is 2½ feet high at the back and 5 feet at the front or north side. This shelter prevents rain from beating the young plants into the ground and provides the shade which is needed after transplanting. A spacing of 4 to 5 feet between beds will allow for passage of laborers and admit adequate light.

The beds are oriented east and west so that a uniform shade is furnished throughout the day without entirely closing in the beds. In Central America, the beds were located without consideration to direction, but were placed on the contour of the land as is shown in figure 6,*C*. Such a practice is probably of value in conserving soil where terrain is steep and rainfall high.

FIGURE 6.—A, Cinchona transplant nurseries in Puerto Rico temporarily sheltered with palm leaves. *B*, Machine used in Costa Rica to open rows in cinchona nursery beds. *C*, Nursery beds built on the contour in Guatemala. Shade for the young plants is obtained by placing strips of wild cane on light wooden frames. *D*, Nursery beds in Costa Rica shaded with elephant grass. *E*, *C. succirubra* and, *F*, *C. ledgeriana* seedlings which will be ready for transplanting to the field in a few months.

Nursery management

SOIL.—According to Sands (*27*), the Dutch planters filled the foot-high beds with fresh jungle mold much as for planting seed. In Puerto Rico a mixture of 2 parts coarse leafmold, 1 part sand, and 1 part topsoil has given good results and seems to provide the necessary drainage and aeration. At one plantation in Guatemala, composted stable manure was added to the nursery soil and seemed to benefit growth.

Investigations at this station have shown that cinchona transplants growing in a soil containing medium to high nitrogen are more tolerant of strong sunlight and environment in general than are poorly nourished plants.

Loustalot and Winters (*14*) showed the young transplants to be particularly sensitive to fertilizer balance. In a controlled experiment the low level of nitrogen had a marked depressing effect on growth, but there was no statistically significant difference in the growth of plants receiving medium and high levels of nitrogen. High levels of phosphorus in a growing media with low nitrogen greatly depressed growth of the cinchona seedlings. A medium supply of phosphorus produced similar but less marked effects. On the other hand, growth of plants in a high nitrogen media was directly correlated with phosphorus level. These results were in agreement with those of Cowgill (*4, pp. 90–104*).

TRANSPLANTING.—The plants are removed from the seedbed with roots bare and are planted 6 inches apart in the nursery. The foliage is not usually trimmed for this first transplanting. In order to prevent lodging it will be necessary to set the plants slightly deeper than they grew in the seedbed. In Costa Rica the device shown in figure 6,*B*, was used to mark rows in the nursery beds for planting.

The transplants should be watered after they are planted to settle the soil around the roots. If the weather becomes dry continued watering may be required at least until the plants become established.

In an experiment conducted under controlled conditions, Loustalot et al. (*15*) showed soil moisture level to be an important factor in growth of *Cinchona ledgeriana* seedlings. Plants with both high and medium soil moisture grew better than plants in low-soil-moisture conditions, but leaves of seedlings growing under high soil moisture showed many necrotic spots which were apparently of a physiological nature. The plants grown with medium soil moisture were more thrifty and considered best for transplanting. This soil-moisture study has a practical application in growing the seedling transplants described in this publication. When transplanted to the nurseries toward the end of the rainy season the young seedlings are protected from excess soil moisture by the palm leaf roof which sheds most of the rain. The following season they are usually transplanted to the plantation before the heaviest rains occur and before the nursery soil remains at or near field capacity for long periods.

If seedlings are transplanted toward the end of the rainy season, it probably will not be necessary to give additional shading except at the ends of the beds. For plantings made during the dry season the sides of the beds are also enclosed with palm leaves for a few days, until the plants recover from transplanting. This protection is the first to be removed when the seedlings become established. Later as the plants develop the top shade is gradually thinned until the plants receive full sunlight.

Plants set in the nurseries during the summer rainy season will be ready for field planting the following year at the beginning of the rainy season. In figure 6,*E* and *F* are shown nurseries of *Cinchona succirubra* and *C. ledgeriana* in Costa Rica during the dry season ready for transplanting as soon as the weather is suitable.

TEMPERATURE.—In a carefully controlled experiment Winters et al. (*34*) found a difference in growth response of two species of *Cinchona* to relatively small differences in temperature. Seedlings of *C. pubescens* grown in a warm environment (70° to 80° F.) had an average dry weight about twice that of plants grown in the cold treatment (60° to 70° F.), while plants grown in the medium temperature environment (65° to 75° F.) were intermediate. On the other hand, *C. ledgeriana* seedlings grew best in the medium temperature and were more exacting in their temperature requirements than *C. pubescens*. Development of root systems of *C. ledgeriana* was retarded by higher temperatures, but the shoot-root ratio of the *C. pubescens* plants was not significantly affected by temperature.

High total alkaloids and quinine in roots and stems of *Cinchona pubescens* seemed to be associated with higher temperatures and vigorous growth. In *C. ledgeriana* there was no consistent trend in regard to these constituents.

WEEDING.—The raw leafmold mixed into the soil usually contains seeds of numerous weeds which germinate freely. These should be removed before they compete with the young cinchona seedlings. Where soil is fumigated for disease control, weed seeds may also be killed.

Asexual Propagation

Cuttings

The use of cuttings, marcottage, and layering, has also been used as a means of propagating the various species of *Cinchona* in Java and India. Moens (*17 p. 149*) states that, "in 1858 multiplication was carried on exclusively in an artificial way because the trees did not yet blossom." Several techniques are described (*17, pp. 156–158*), but the most successful method seems to have been by the use of glass-covered frames over sand beds in the greenhouse, or in shaded beds in the open during the rainy season. He considered cuttings from mature Ledgers difficult to root.

The cuttings were secured by topping plants in open beds and using the sprouts which formed, as cuttings. When rooted, the cuttings were planted in small pots until well established, then shifted to outdoor nurseries. Using this method there was little difference in the rooting of *Cinchona ledgeriana*, *C. succirubra*, and *C. officinalis*. Layering and marcottage were used mostly in the *C. succirubra* plantations of British India. Recent work in India by Guha Thakurta and Dutt (*9*) has shown that air-layers of *C. ledgeriana* can be rooted in 4 months by treatment with 2-percent indolebutyric acid in lanolin paste. The authors claim vigorous root formation in 88 percent of the air-layers and suggest the method for propagation of high-yielding stocks.

In Guatemala and Costa Rica, high alkaloid-yielding strains of *Cinchona succirubra* are propagated by cuttings. The stock plants are bent over horizontally and pegged down as is shown in figure 7,*C*. The shoots which are freely produced from the base and nodes of the trunk are cut off when of sufficient size and rooted in sand beds. A bed of the plants almost ready for transplanting to the field is shown in

figure 7,*D*. This method of propagation is particularly useful for propagating select or high-yielding strains of *C. succirubra*.

Results of rooting trials conducted in Puerto Rico (*32*, *pp. 16–17*) indicate a great difference in the rooting ability of *Cinchona ledgeriana*

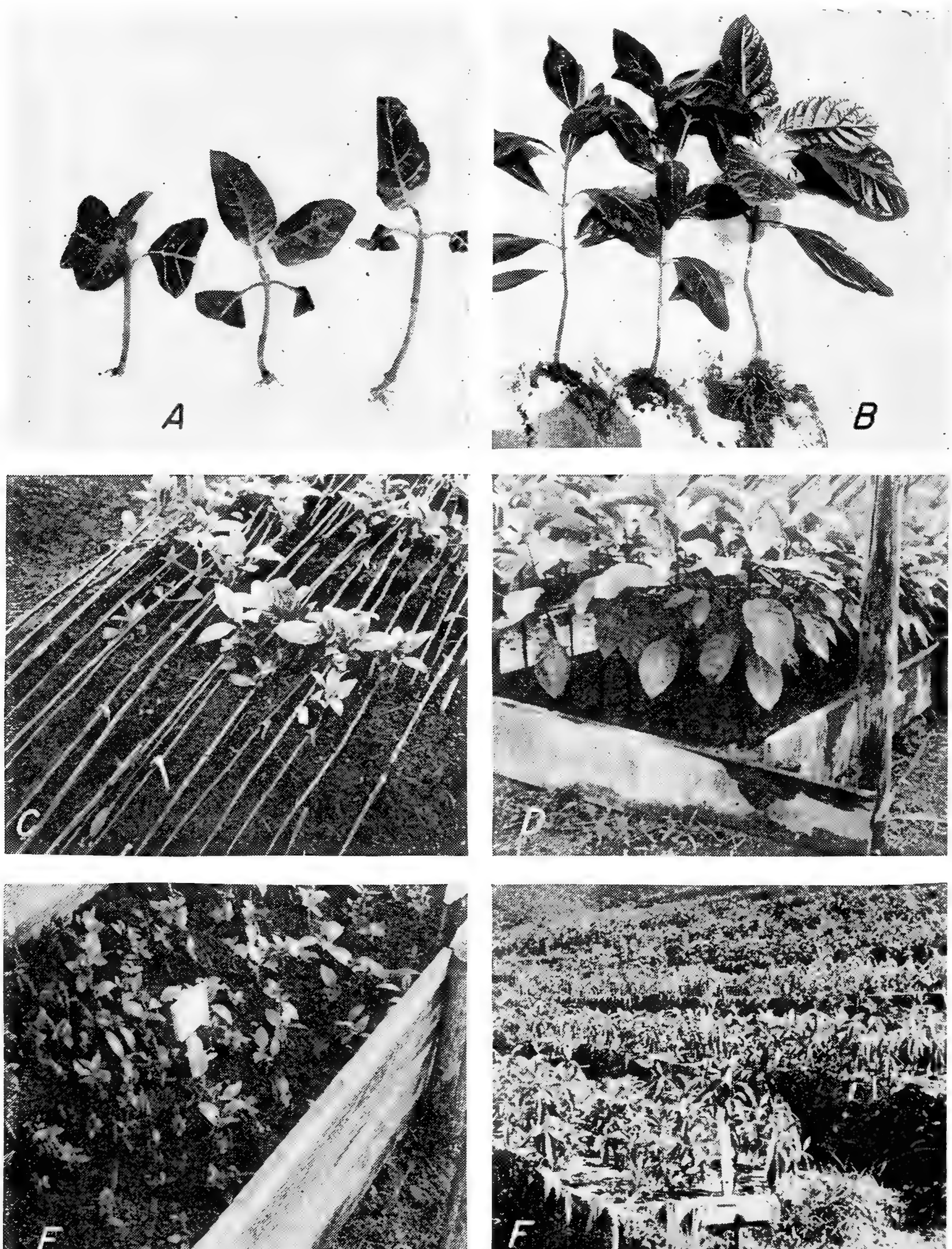

FIGURE 7.—*A*, Cuttings of *Cinchona succirubra* root readily in sand. *B*, Two plants of *C. ledgeriana* (left) and one plant of *C. succirubra* (right) which were grown from cuttings. *C*, In Guatemala, these plants of a high-alkaloid strain of *C. succirubra* were placed horizontally in order to induce a crop of sprouts which will be used for cuttings. *D*, *C. succirubra* plants grown from cuttings ready for field planting. *E*, Bench grafts of *Cinchona* heeled in nursery frame. After stocks and scions are united they will be planted in nurseries. *F*, Nurseries of *C. ledgeriana* grafts a few months after grafting.

and *C. pubescens* (*C. succirubra*) cuttings from mature trees. With the *C. ledgeriana* cuttings there appeared to be an indirect correlation between age of plants from which the cuttings were taken and their ability to root. Best results were obtained in rooting cuttings from young seedlings (fig. 7,*A*), whereas cuttings taken from mature trees failed to root at all. On the other hand, cuttings of young wood from mature trees of *C. succirubra* rooted fairly well. Cinchona plants grown from cuttings (fig. 7,*B*) usually have well-developed root systems.

Grafting

Since propagation of high alkaloid cinchona strains by artificial means is necessary to insure an equally high alkaloid content in the progeny, the Dutch planters early resorted to grafting (*17, p. 159*). The method employed was bench grafting, using the side graft on *Cinchona succirubra* rootstocks. The only difference in the two methods was that for bench grafting the plants were lifted from the nurseries and the grafting operation performed in greenhouses or other controlled environments. More recently, the Dutch planters have followed the practice of grafting in outdoor nursery beds described by Sands (*27*), Blücher (*1*), and others. In Guatemala both methods are in use (fig. 7,*E* and *F*). During the rainy season grafting is commonly performed in open nuseries as reported by Pinkus (*20*). The grafting season is also extended by employing the bench grafting method during dry weather. In the field a modification of the side or veneer graft is universally used. In bench grafting either this method or cleft grafting is used.

In bench grafting, as performed in Guatemala, the *Cinchona succirubra* rootstocks are lifted from the nurseries and transported bare-rooted to wooden houses with earth floors where the grafting operation is performed. The grafted plants are arranged close together on the dampened floor and left for about 2 weeks by which time the union will have been accomplished. They are then removed and planted in nursery beds in the open but heavily shaded on the sides and top. Bench grafting can be used to utilize rootstocks on which the grafts made during the rainy season failed to take.

Moens (*17, p. 161*) reported that the cleft graft did not give as good results as the side graft. The plants did not grow well and produced a large swelling at the graft union. This is probably due to a growth differential between the vigorous *Cinchona succirubra* rootstocks and the *C. ledgerinana* tops. This trouble has not been reported from Guatemala where the clones used for grafting are apparently hybrids and almost as vigorous in growth as the *C. succirubra* rootstocks.

DISEASES AND INSECTS

In handling, mixing, and applying poisonous fungicides and insecticides for the control of disease or insects special care should be taken not to inhale dusts or sprays at any time. Well-designed respirators that afford protection to the entire face should be kept handy and used when such danger exists. After working with insecticides, the hands or any exposed parts of the body should be washed thoroughly with soap and water.

Containers in which these materials are kept or stored should be plainly labeled and placed under lock and key, or at least out of the reach of children and others unfamiliar with their poisonous nature. Any unused portions of these poisonous preparations as well as the receptacles in which they have been mixed should be treated likewise.

Diseases

A considerable number of cinchona diseases have been reported particularly from Asia where the crop has been most extensively cultivated. Diseases are important in American cinchona culture also and in some sections they are probably the limiting factor in development of this crop. An excellent review of the world literature on diseases in cinchona has been prepared recently by Lombard (*13*).

Seedbed Diseases.—The term "damping-off" is used to describe any fungus infection which causes the rapid decay or death of young seedings (*13, pp. 2–3*). In Puerto Rico it is thought to be caused by a basidiomycete (*26, p. 43*), although the casual organism has not been identified and other fungi may also be involved.

As stated previously (p. 11) careful attention to watering is a good means of preventing damping-off. Additional indirect measures for controlling this disease are: The use of new soil with each planting of seed and attention to time of seeding. According to Popenoe (*24*) *Cinchona* in Guatemala is rarely attacked by damping-off if the seed is sown at the beginning of the dry season and the plants are several months old before the rainy season starts. However, there are times during periods of unfavorable weather when disease may appear in the seedbeds, as is shown in figure 5,*A*. Stoddard (*30, p. 29*) found that liquid applications of Semesan and Cuprocide following germination of the cinchona seed controlled spread of the disease. Treatment of the seed with these materials as dusts before planting, however, reduced the stand of seedlings by 90 percent or more. Reinking [4] reports that solutions of bordeaux mixture and Yellow Cuprocide have been used to control damping-off in cinchona seedbeds in Guatemala. Caution must be used in the application of chemicals as the young seedlings are more than ordinarily sensitive.

In Puerto Rico damping-off has been prevented in cinchona seedbeds by fumigation of the soil with chloropicrin previous to seeding. The chemical was injected at the rate of 2 milliliters per square foot of soil surface. When treated soil was surfaced with 1 inch of sphagnum moss growth of the plants was stimulated and damping-off was eliminated.

Nurseries.—Damping-off disease, which sometimes causes extensive damage to the cinchona seedbeds, may also occur in the transplant nurseries. It is not common, however, after the plants become established and can usually be prevented where watering can be regulated so that the surface of the soil is dry at night.

The diseases most prevalent in the cinchona nurseries in Puerto Rico are stem blight, cankers, and root rots. Stem blight which has

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[4] Reinking, D. A. Report on cinchona diseases in Guatemala. U. S. Bur. Plant Indus., Soils, and Agr. Engin., Plant Disease Rptr. 29: 432–439. 1945. [Processed.]

been troublesome only in the nurseries in Puerto Rico appears to be the same *Phytophthora* disease reported by Crandall and Davis (*6, p. 138*) on plantation trees in Central and South America. It is characterized by a rather sudden blighting of the young foliage and stems at the growing tips of the plants as is shown in figure 5,*B*. The initial infection occurs at the base of the leaf blade and travels downward through the petiole to the stem. The young plant may be killed immediately or may recover sufficiently to produce a new shoot from healthy tissue below the blighted tip, as shown in figure 5,*C*. Such plants are not considered suitable for transplanting to the field. The disease attacks both *Cinchona ledgeriana* and *C. succirubra*.

A more prevalent and somewhat similar disease occurs on *Cinchona* in Puerto Rico· It is thought that the causal organism may be the same as that causing the tip blight. In this disease the foliage may be observed to wilt during the warmest part of the day. Investigation reveals a canker running from the root zone to part way up the stem. Usually one or more of the large roots is dead. These cankers generally do not extend more than one-fourth around the circumference of the stem. At first they are not readily seen but later appear sunken as the healthy part of the stem expands. Plants attacked by this disease do not usually die immediately. In fact, the cankers are not easily seen at first and diseased plants may be transplanted to the field. Such plants may appear to recover from the disease, but the root systems are never well developed and the trees are readily blown over in a windstorm.

Another prevalent disease of cinchona seedlings in Puerto Rico is a root and collar rot with symptoms somewhat similar to the disease caused by *Phytophthora quininea* Crandall (*5*) from Peru. This disease apparently gains entrance through the roots or collar region. The leaves first acquire a reddish appearance, which is followed by wilting and then the shedding of all but the young terminal leaves. The collar region is usually swollen above the dead root system. Plants in this condition may remain alive for several months and often produce new roots from the collar region. The organism causing the disease in Puerto Rico has not been identified. As is shown in figure 5,*D*, there is considerable difference in resistance to this disease between different strains of *Cinchona ledgeriana*. One strain from the Philippine Islands was severely attacked while a vigorous strain originating from a tree in the Maricao planting appeared to be partially resistant.

The symptoms of a second unknown root disease are similar to those of the "gray root fungus" of Java cinchona plantings caused by the *Graphium* or imperfect stage of a species of *Rosellinia* (*12, pp. 9–10*). It has been most troublesome in permanent plantings. The trees appeared healthy but made poor growth. The leaves wilted suddenly, then dried out and remained attached to the tree for some time. It was observed that the main roots had rotted, leaving only short stubs as is shown in figure 5,*E*. Adventitious roots had developed, but when these were attacked by the disease the leaves wilted and died.

Some of the experimental field plantings of *Cinchona* in Puerto Rico have been relatively free of disease but considerable losses have been encountered in most. The diseases apparently are the same as

those causing damage in the nurseries and in many instances may have been carried from the nurseries to the field with the plants.

CONTROL.—Control measures are mostly preventive rather than curative. It is essential to follow rigid sanitation practices in the nurseries and fields. All diseased material should be removed and burned.

Good soil drainage in the nurseries is essential to the healthy growth of seedlings. On several occasions excessive death of seedlings in certain areas of the nurseries has been attributed to poor drainage. It is essential that the nurseries be located in well-drained areas. In addition, drainage can be improved by building the nurseries 6 to 12 inches above the ground level.

STERILIZATION.—In some instances plantings have been made on soil unsuitable for *Cinchona* such as poorly drained clays. In such cases the soil was no doubt a contributing factor to disease.

Treatment of nursery soils with fumigants has proved beneficial in improving survival and preventing fungus diseases of cinchona seedlings under conditions found in Puerto Rico (*33*).

A replicated experiment was conducted to compare the effectiveness of five fumigants. Of these only chloropicrin and D–D (1–3,dichloropropene, 1–2,dichloropropane) at the rate of 300 pounds per acre significantly reduced the count of diseased plants in the nursery beds. Doses of 2 cubic centimeters of both materials were injected into the soil at 8-inch spacing and at two-thirds the soil depth. After application of the chemicals the soil was sprinkled with water and the beds covered with wax paper. After 1 week the paper was removed and the soil was exposed to the air for 2 weeks before planting.

Extreme care should be practiced by the user of chloropicrin and D–D.

Insects

In the course of investigations with *Cinchona* in Puerto Rico, a number of insect and other animal pests have been encountered. As reported previously (*21*) some of them caused serious injury, particularly during early stages of growth.

The greatest injury during the seedbed stage of growth is caused by two species of thrips, *Scirtothrips longipennis* Bagn. and *Anaphothrips* (*Chaetanaphothrips*) *orchidii* (Moult.). Injury appears as a rasping of the upper surface of the leaves, particularly along the midrib and large veins, as shown in figure 8,*A*. At first the damaged tissues turn silvery white. When the damage is extensive (fig. *8,C*), the leaves turn brown and fall off. These insects are most troublesome during the latter part of the dry season. Weekly applications of a 1-percent rotenone dust gave good control. After the plants are transplanted to the nursery beds, thrips injury usually subsides except during the dry weather or while the beds are heavily shaded with palm leaves.

Another species of thrips, *Heliothrips haemorrhoidalis* (Bouché), has caused considerable damage at times during the later nursery stages and after the plants are set in the field. As indicated in figure 8,*B*, this insect feeds largely on the undersides of the leaves. A dust containing 1-percent rotenone has given good control of this pest when applied at weekly intervals. In addition a spray containing 25-percent

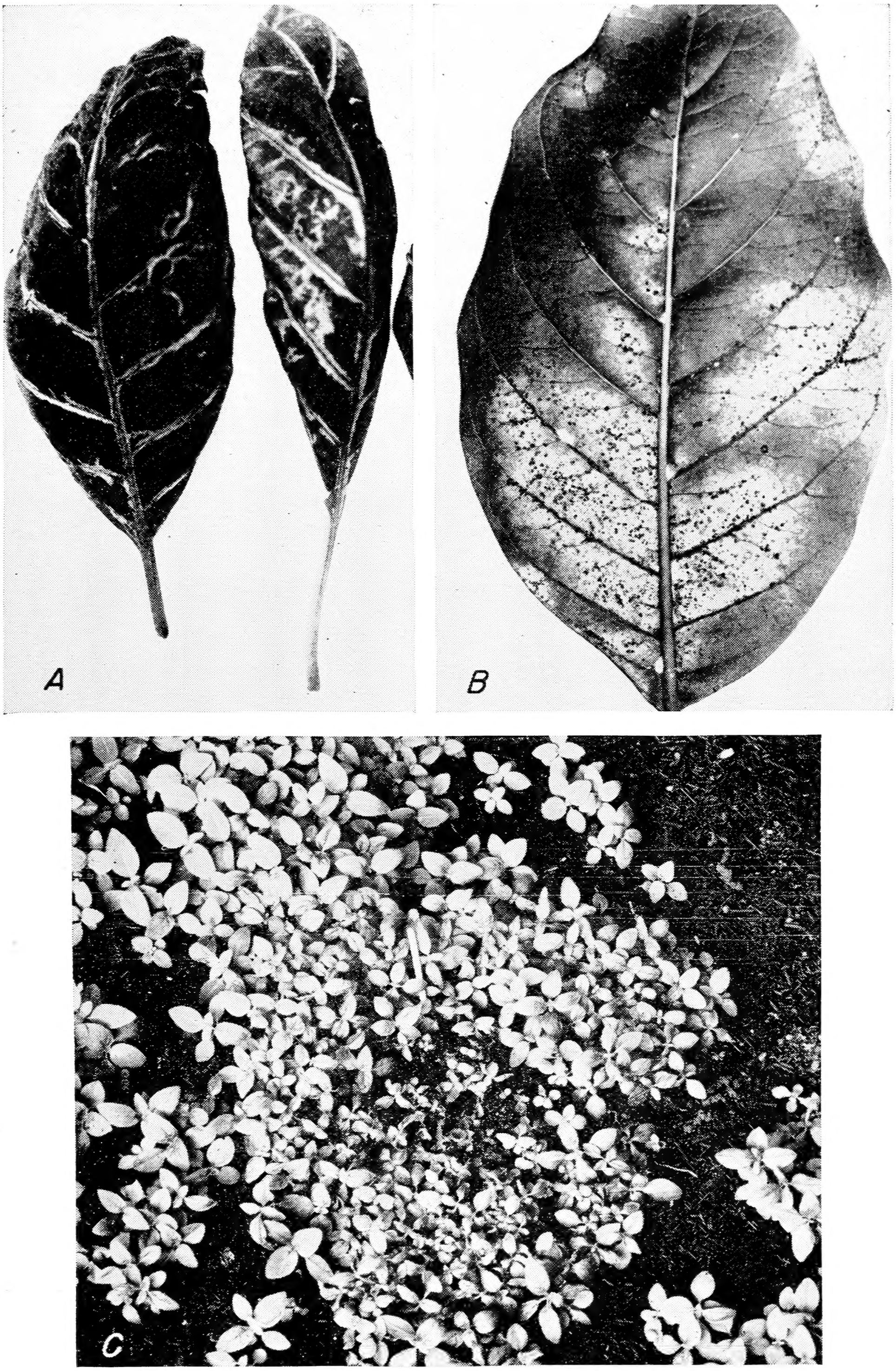

FIGURE 8.—*A*, Characteristic injury caused by feeding of *Scirtothrips longipennis* and *Anaphothrips* (*Chaetanaphothrips*) *orchidii* on leaves of year-old seedlings of *Cinchona succirubra*. The scarred tissue along the veins and the curled leaves are typical of this injury. *B*, Typical injury caused by the feeding on old leaves of *C. calisaya* by *Heliothrips haemorrhoidalis*. *C*, Typical injury to young plants of *C. ledgeriana* caused by *S. longipennis* and *A. orchidii*. Note withered leaves on plants toward the center of the picture.

wettable DDT powder at the rate of 4 pounds to 100 gallons of water has given good control. This solution caused injury to young seedlings but older plants in the nursery and 4-year-old trees in the field were free from injury.

Spider mites (*Tetranychus* sp.) have been serious pests on the underside of the leaves of small cinchona seedlings in the greenhouse at Mayaguez and in the nurseries at Maricao. The mites appear during periods of dry weather and may cause considerable damage to the small seedlings if not checked. Complete control has been obtained with dusting sulfur.

Scale insects of several species have been found at various times on *Cinchona*. The most prevalent has been the green scale (*Coccus viridus* (Green)), which attacks the leaves and tender stems of the plants. This insect has been troublesome both in the nurseries and in field plantings. Partial control occurs through a parasitizing fungus, probably *Hypocrella* sp., but apparently not until the young plants have been damaged.

The mining scale (*Howardia biclavis* (Comst.)) has also damaged plants in the nurseries and field plantings at Maricao. Injury was particularly severe on *Cinchona ledgeriana*.

Several lepidopterous and orthopterous insects also have been found feeding on cinchona plants in Puerto Rico. It is though that such feeding is mostly casual, the insects having wandered onto the cinchona plants from surrounding vegetation. The most common of these casual feeders are several species of *Dyme* or walking stick insects which are particularly abundant on natural vegetation in the Toro Negro area.

Leaf-cutting ants have been troublesome at times on *Cinchona ledgeriana* (fig. 9) in Central America, but have not bothered the plants in Puerto Rico. The remedy is to track the ants to the nest and exterminate them with carbon tetrachloride.

FIGURE 9.—Injury to young plants of *Cinchona ledgeriana* by leaf cutting ants.

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